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"Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation. Design of Pyrimidine Specific Bases"

by

L. C. Griffin, L. L. Kiessling and P. B. Dervan

California Institute of Technology
Division of Chemistry and Chemical Engineering
Pasadena, CA

June 1, 1991

Recognition of All Four Base Pairs of Duplex DNA by Triple Helix Formation. Design of Pyrimidine Specific Bases.

The novel base 4-(3-benzamido)phenylimidazole was designed, synthesized and incorporated within a pyrimidine oligonucleotide and shown to recognize pyrimidine·purine base pairs over purine·pyrimidine base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in simian virus 40 DNA containing *all four base pairs* at physiologically relevant conditions.

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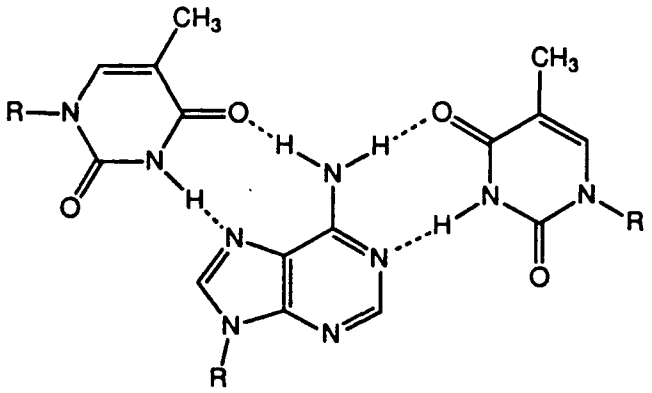
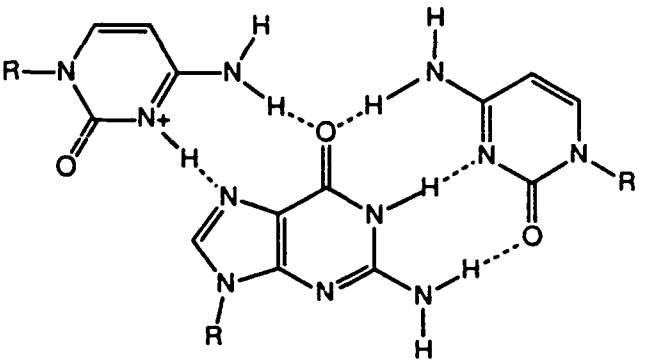
*L. C. Griffin, L. L. Kiessling and P. B. Dervan**

Arnold and Mabel Beckman Laboratories of Chemical Synthesis
California Institute of Technology
Pasadena, California 91125

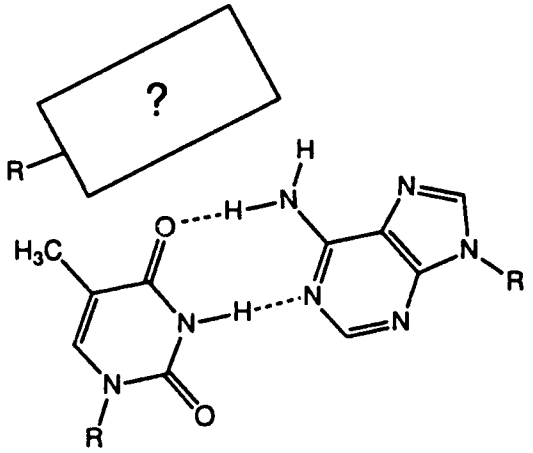
A novel nucleotide equipped with the base, 4-(3-benzamido)phenylimidazole, was designed, synthesized and incorporated within a pyrimidine oligonucleotide. This was shown to recognize CG and TA base pairs over GC and AG base pairs. Such specificity allows binding by triple helix formation at an 18 base pairs site in simian virus 40 DNA containing all four base pairs at physiologically relevant conditions.



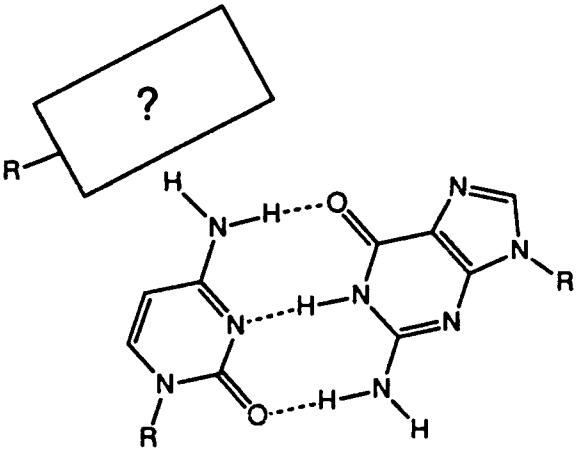
T•AT



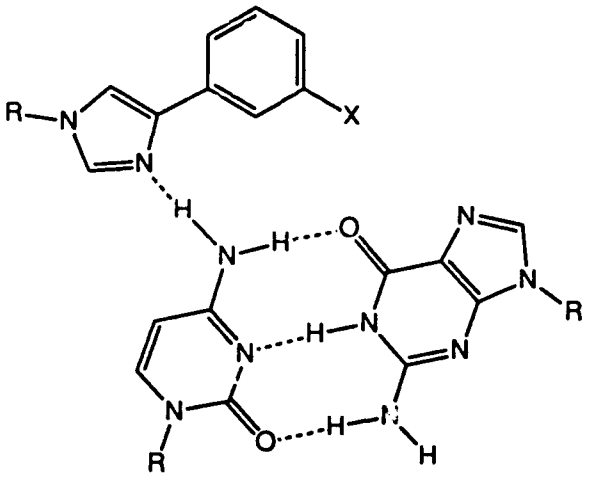
C+GC



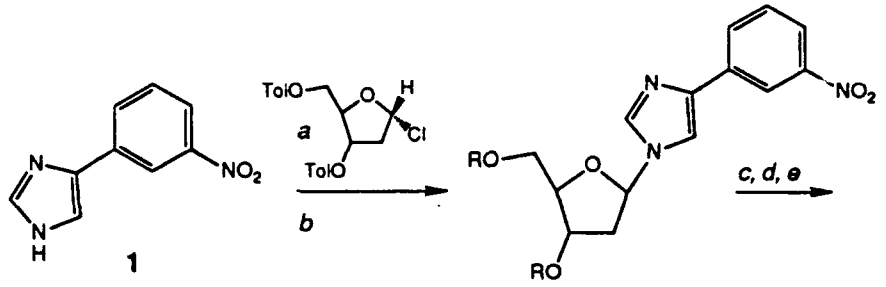
?•TA



?•CG

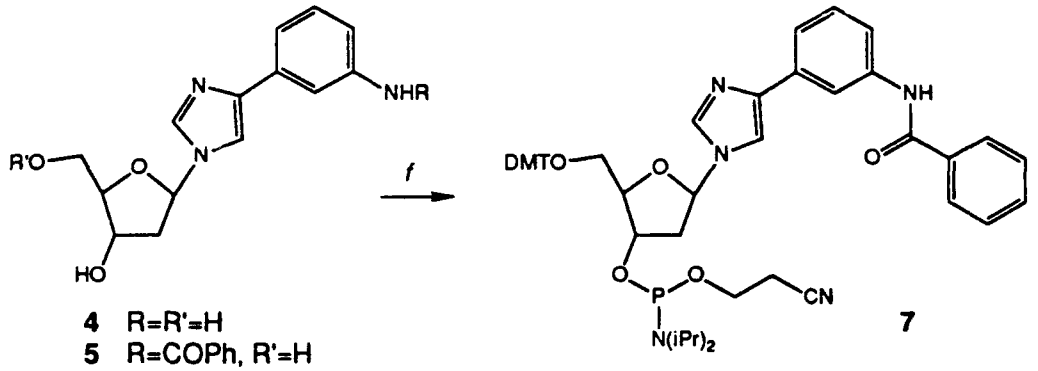


D_1 X=H
D_2 X=NH_2
D_3 X=NHCOPh



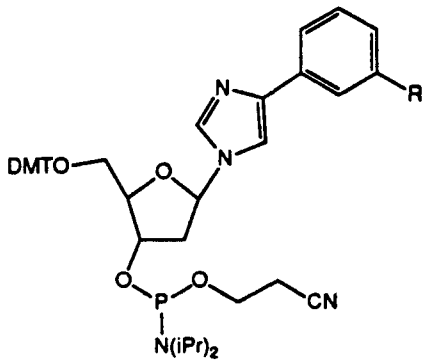
1

2 R=Tol
3 R=H



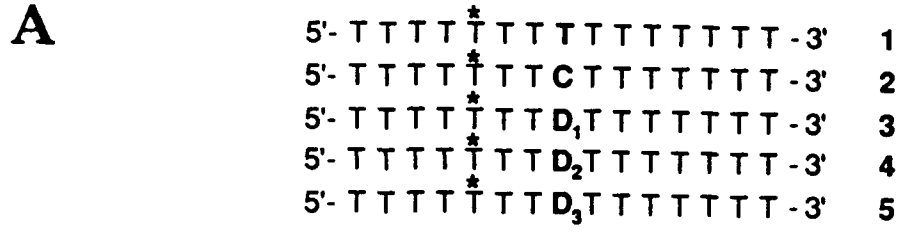
4 R=R'=H
5 R=COPh, R'=H
6 R=COPh, R'=DMT

7



8 R = H
9 R = $NHCOCF_3$

A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5'- T T T T T T T T C T T T T T T - 3' 2
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5'- T T T T T T T T D2 T T T T T T - 3' 4
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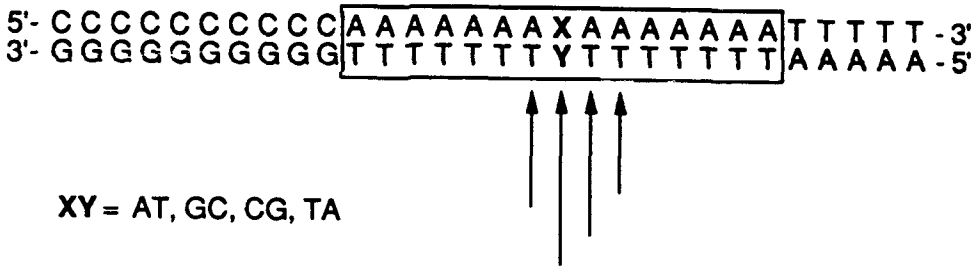
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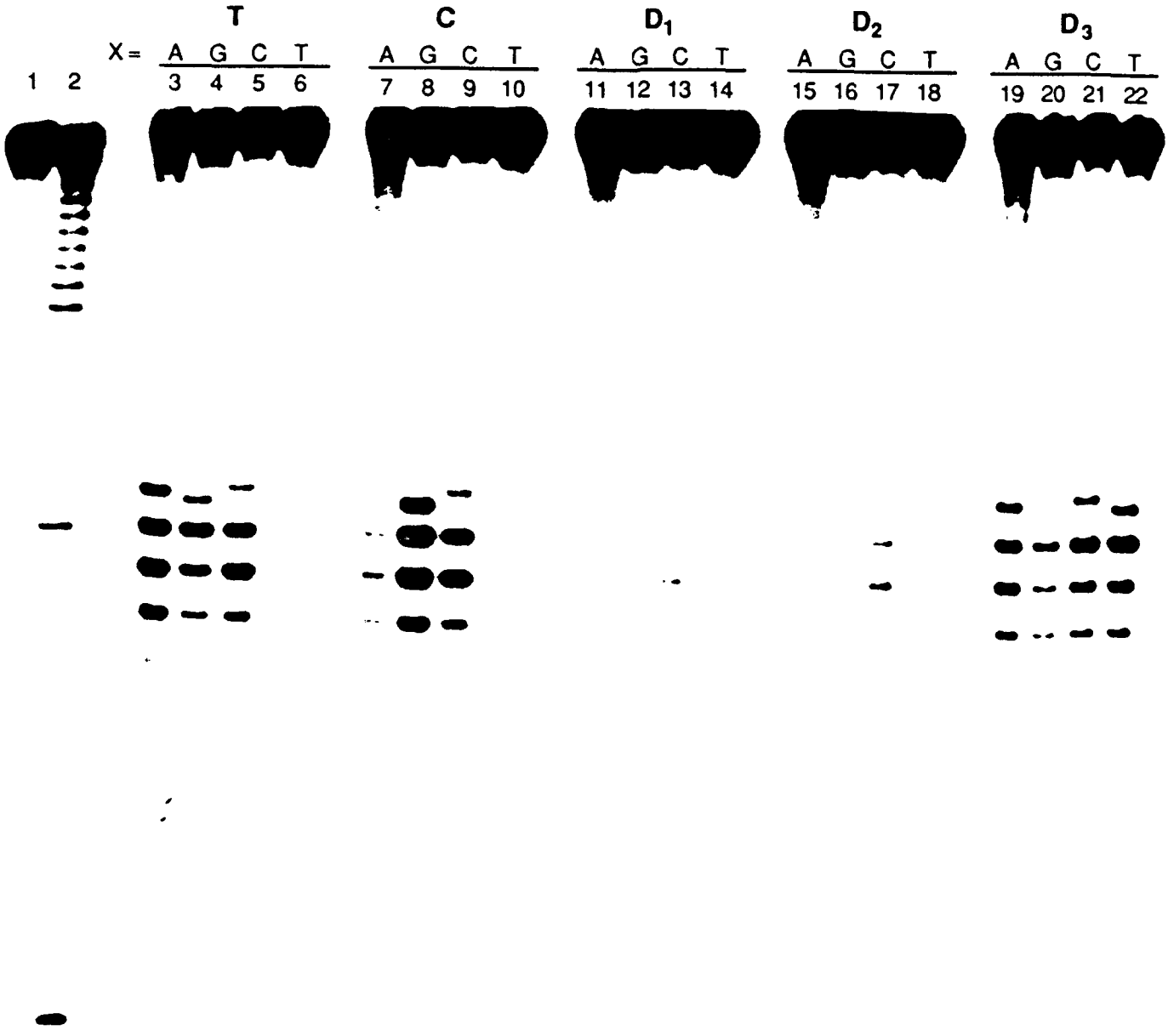
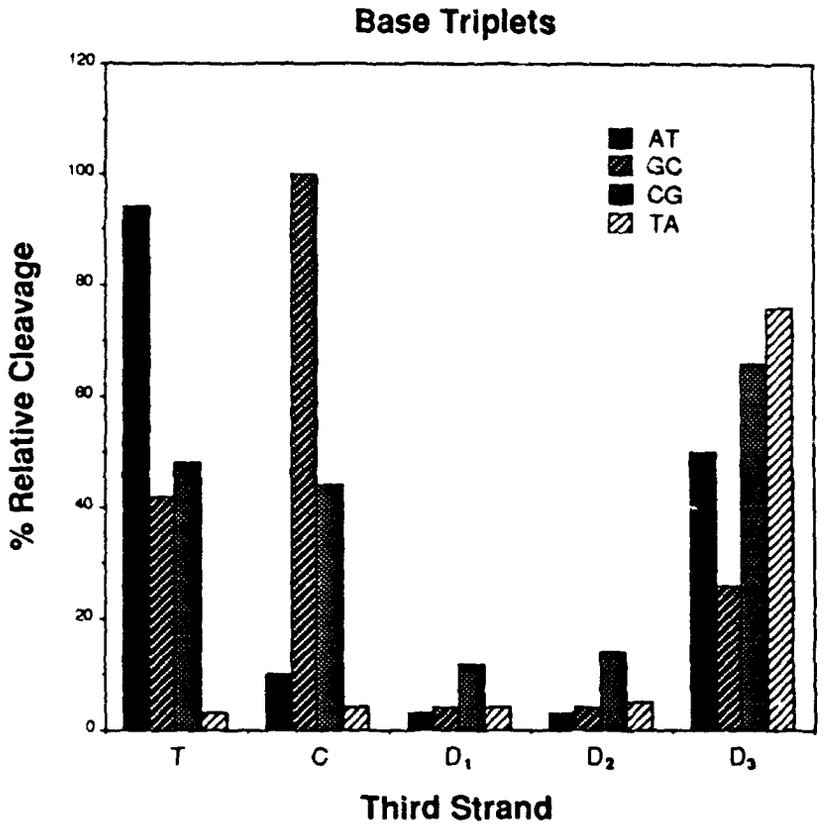
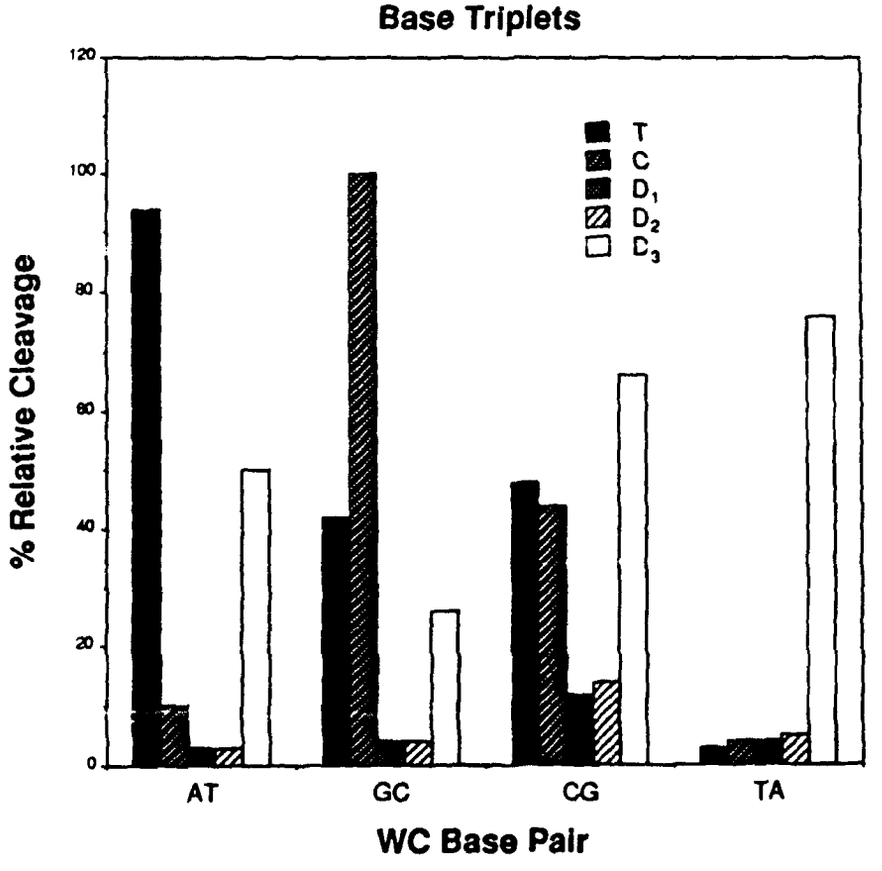
3'- G G G G G G G G G G T T T T T T T Y T T T T T T T A A A A A - 5'
XY = AT, GC, CG, TA

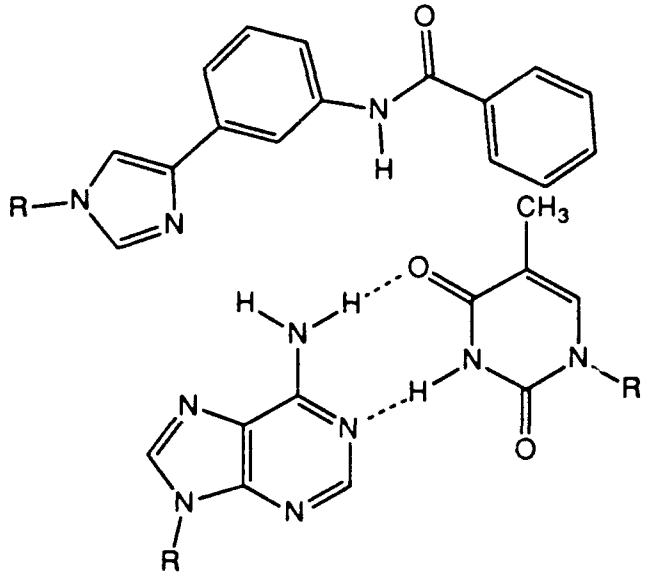

T
C
D1
D2
D3
X= A G C T
A G C T
A G C T
A G C T
A G C T
1 2
3 4 5 6
7 8 9 10
11 12 13 14
15 16 17 18
19 20 21 22

C

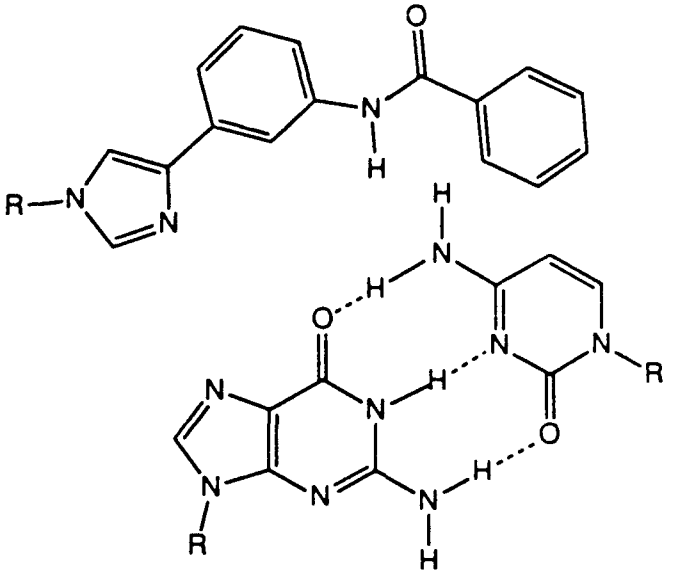

Base Triplets
120
100
80
60
40
20
0
% Relative Cleavage
AT
GC
CG
TA
T
C
D1
D2
D3
Third Strand


Base Triplets
120
100
80
60
40
20
0
% Relative Cleavage
T
C
D1
D2
D3
AT
GC
CG
TA
WC Base Pair

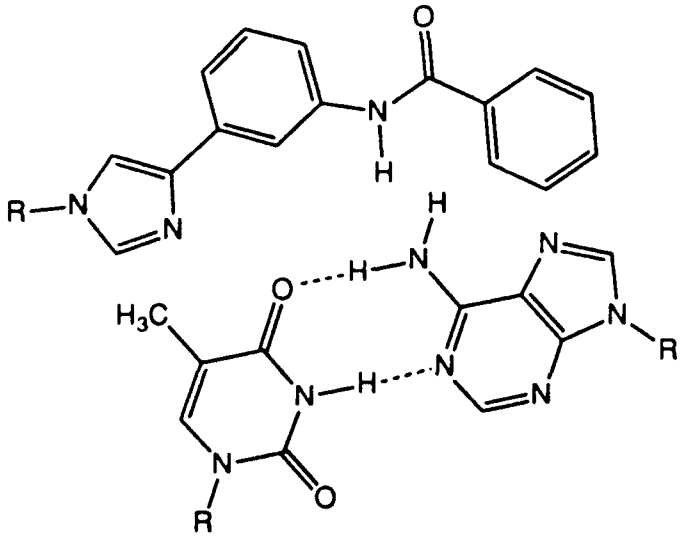
D



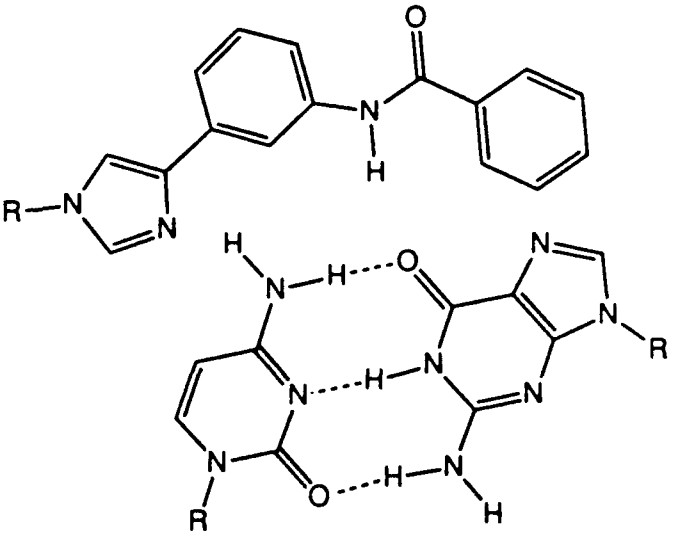
D_3•AT



D_3•GC



D_3•TA



D_3•CG

A

5'- T T T T T T T T C T T T T T T T C T -3' 6
5'- T T T G T T T T C T D_1 T T T T T T C T -3' 7
5'- T T T G T T T T C T D_2 T T T T T T C T -3' 8
5'- T T T G T T T T C T D_3 T T T T T T C T -3' 9
5'- T T T D_1 T T T T C T D_1 T T T T T T C T -3' 10
5'- T T T D_3 T T T T C T D_1 T T T T T T C T -3' 11
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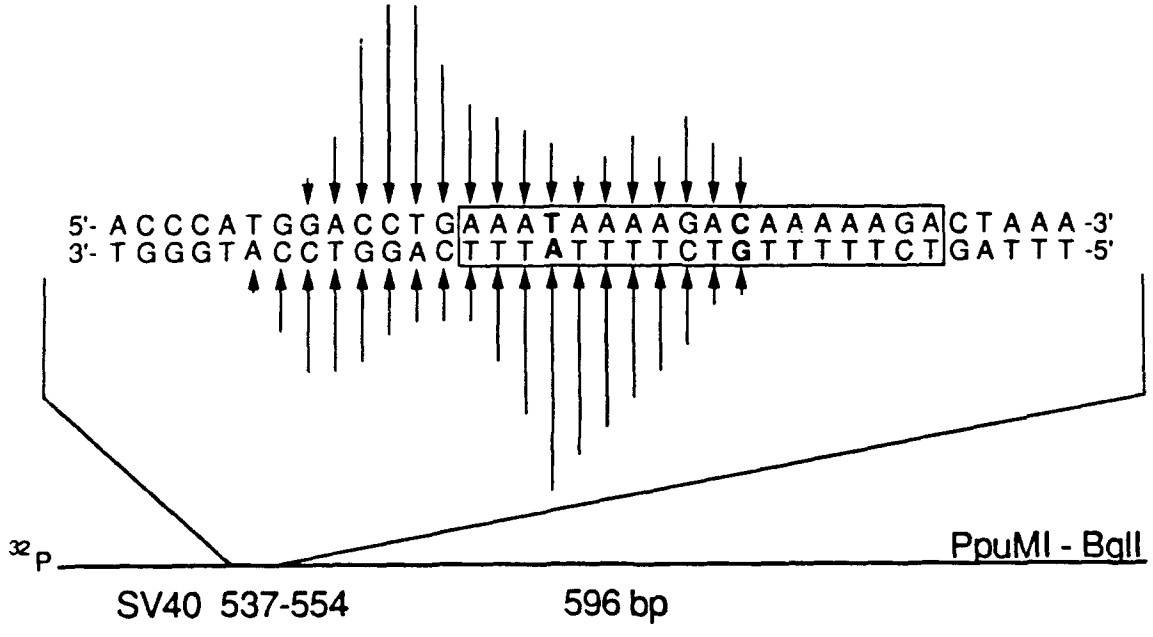
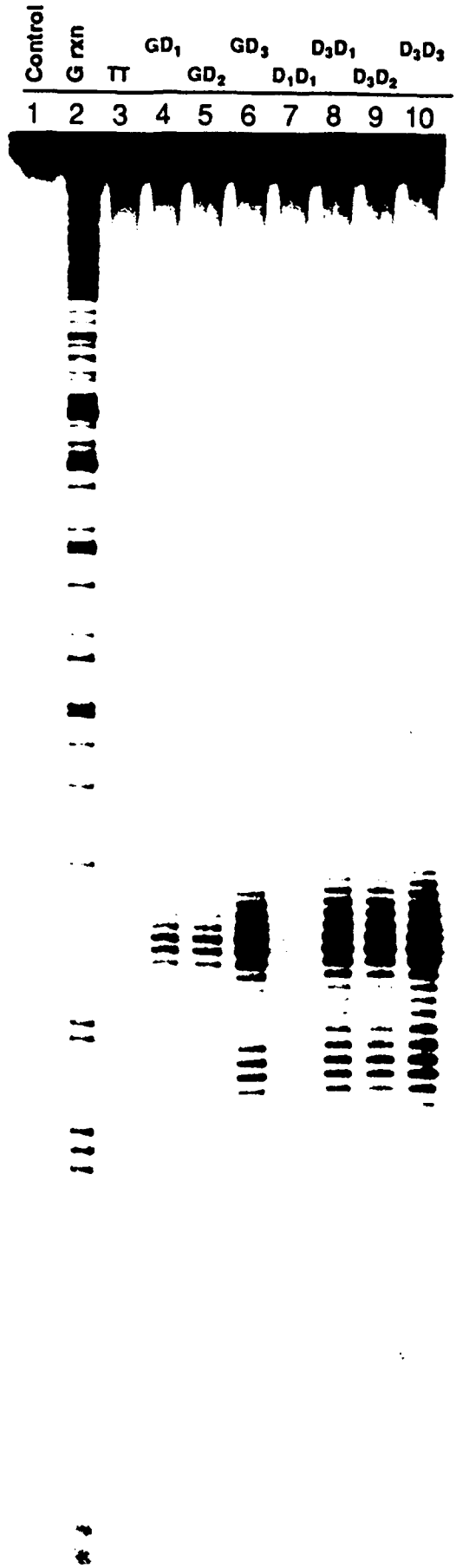
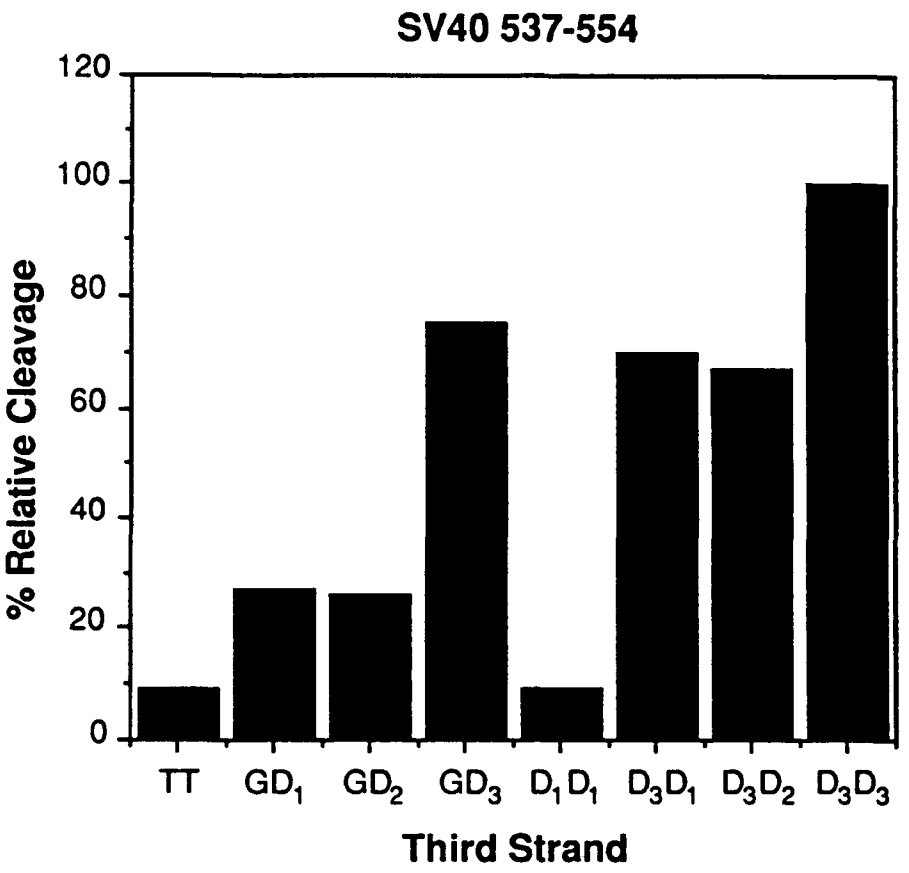
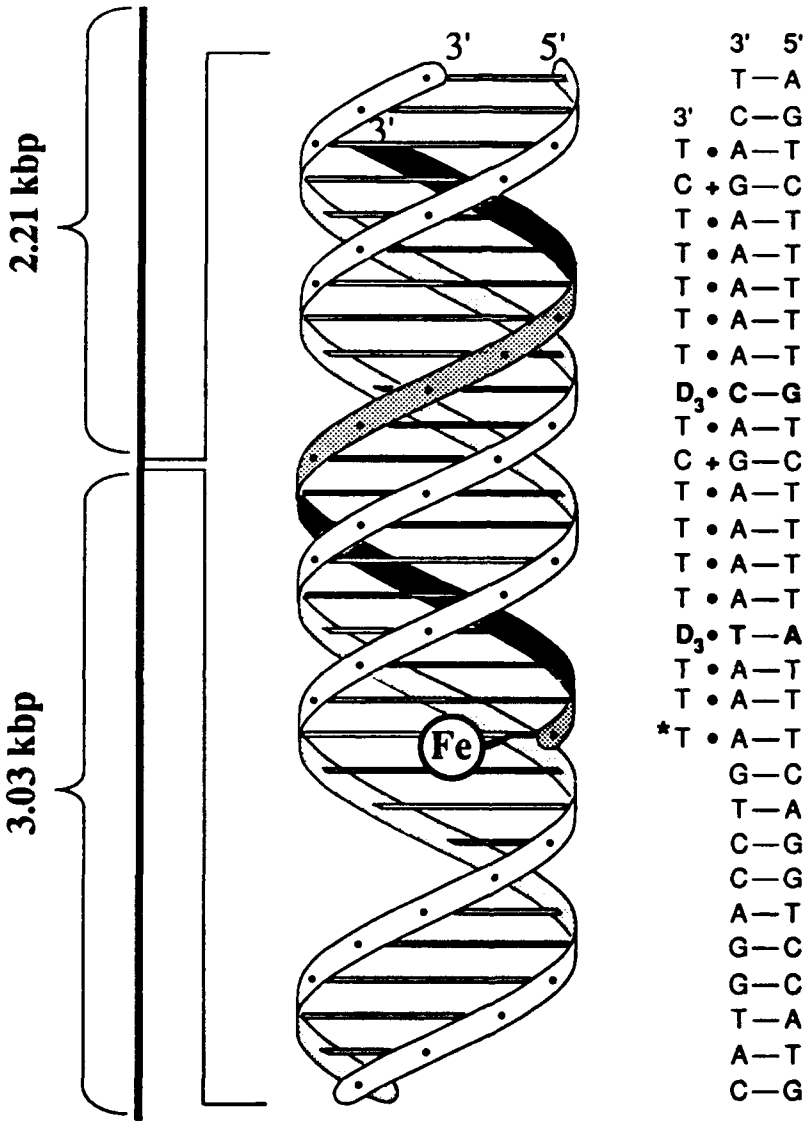
5'- T T T D_3 T T T T C T D_3 T T T T T T C T -3' 13

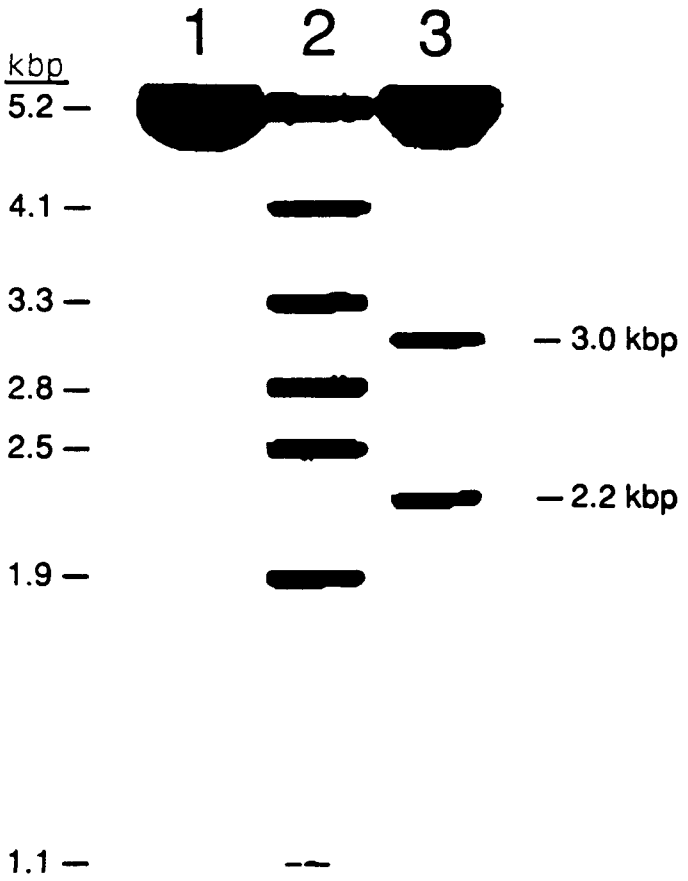



Control
G rxn
TT
GD1
GD2
GD3
D1D1
D3D1
D3D2
D3D3
1 2 3 4 5 6 7 8 9 10

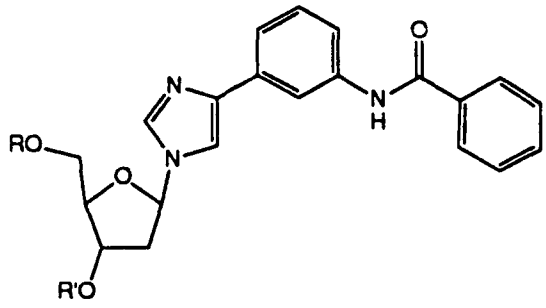
C


SV40 537-554
120
100
80
60
40
20
0
% Relative Cleavage
TT
GD1
GD2
GD3
D1D1
D3D1
D3D2
D3D3
Third Strand

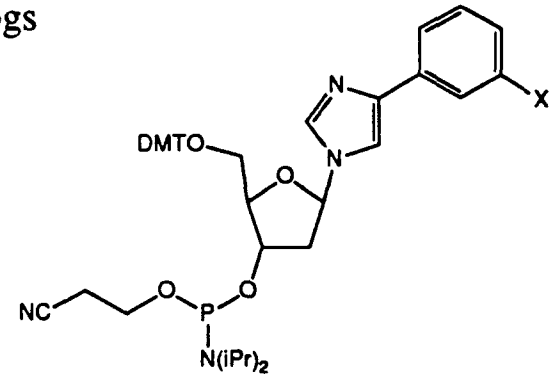

2.21 kbp
3.03 kbp
3'
5'
3'
Fe
3' 5'
T—A
3' C—G
T • A—T
C + G—C
T • A—T
T • A—T
T • A—T
T • A—T
T • A—T
D3 • C—G
T • A—T
C + G—C
T • A—T
T • A—T
T • A—T
T • A—T
D3 • T—A
T • A—T
T • A—T
*T • A—T
G—C
T—A
C—G
C—G
A—T
G—C
G—C
T—A
A—T
C—G

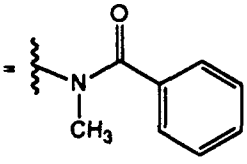

1
2
3
kbp
5.2
4.1
3.3
2.8
2.5
1.9
1.1
3.0 kbp
2.2 kbp

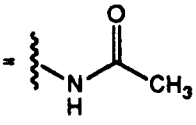
Design Lead: D_3

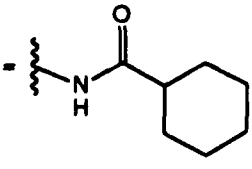


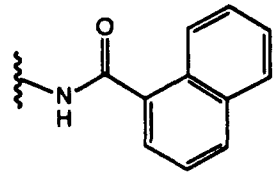
Base Analogs

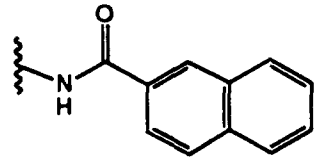


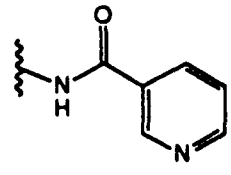
D_4 (10): X = 

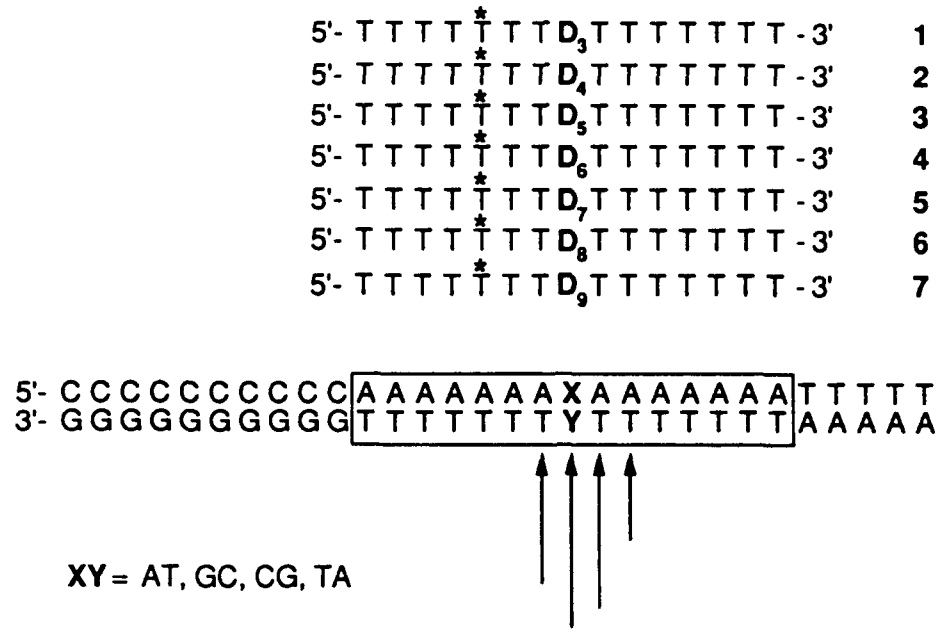
D_5 (11): X = 

D_6 (12): X = 

D_7 (13): X = 

D_8 (14): X = 

D_9 (15): X = 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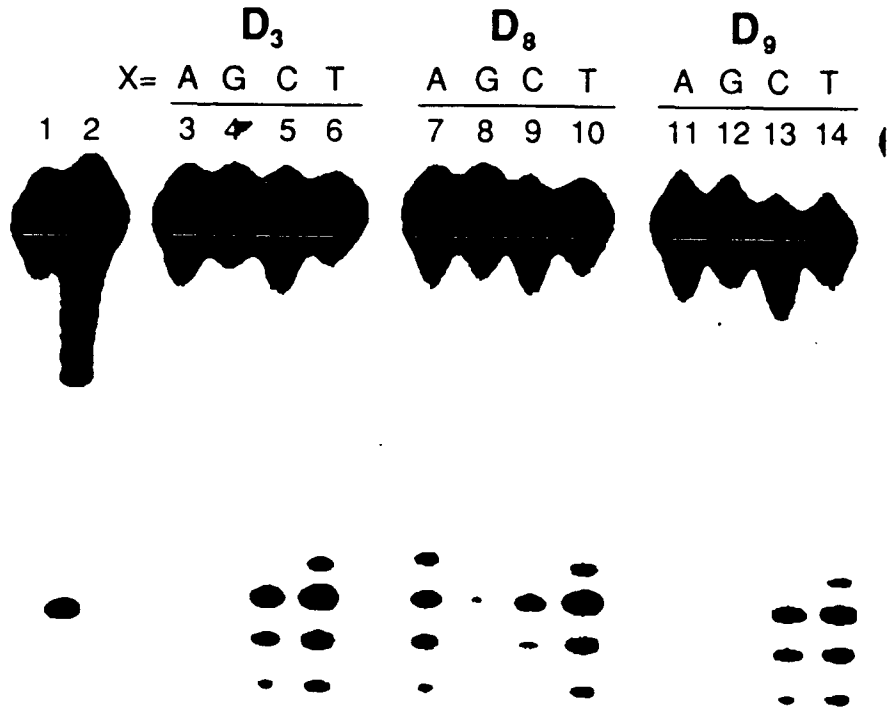
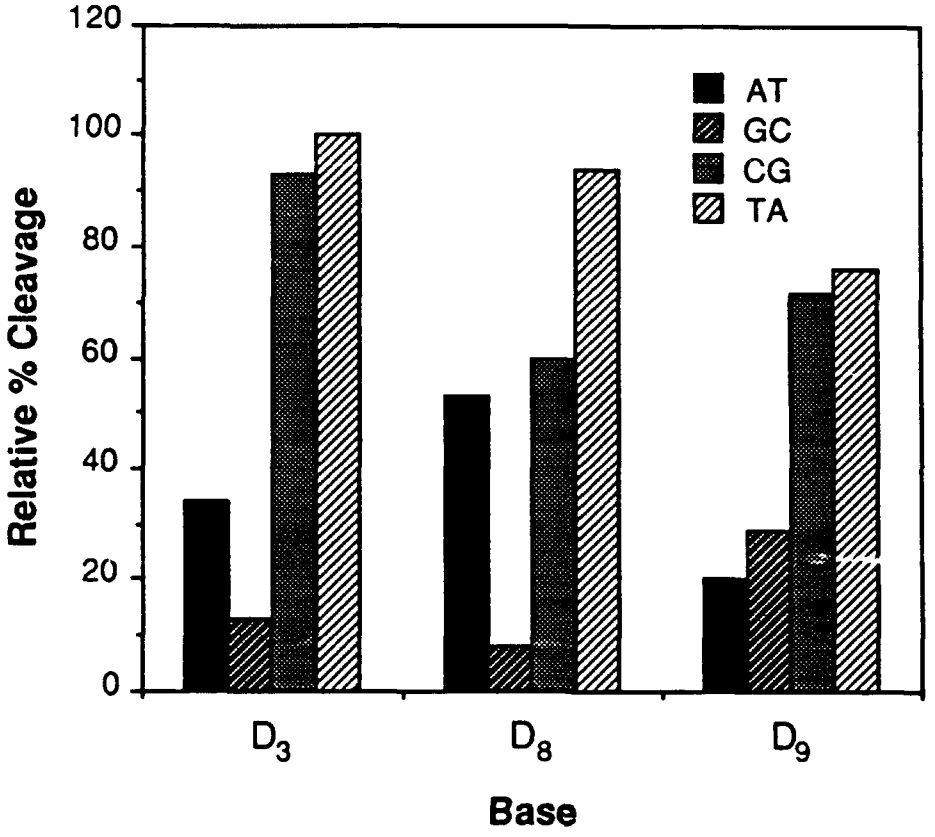
5'- T T T T T T T T D6 T T T T T T T T - 3' 4
5'- T T T T T T T T D7 T T T T T T T T - 3' 5
5'- T T T T T T T T D8 T T T T T T T T - 3' 6
5'- T T T T T T T T D9 T T T T T T T T - 3' 7
5'- C C C C C C C C C C C A A A A A A A X A A A A A A A T T T T T - 3'
3'- G G G G G G G G G G G T T T T T T T Y T T T T T T T A A A A A - 5'
XY = AT, GC, CG, TA


D3
D8
D9
X= A G C T
A G C T
A G C T
1 2
3 4 5 6
7 8 9 10
11 12 13 14

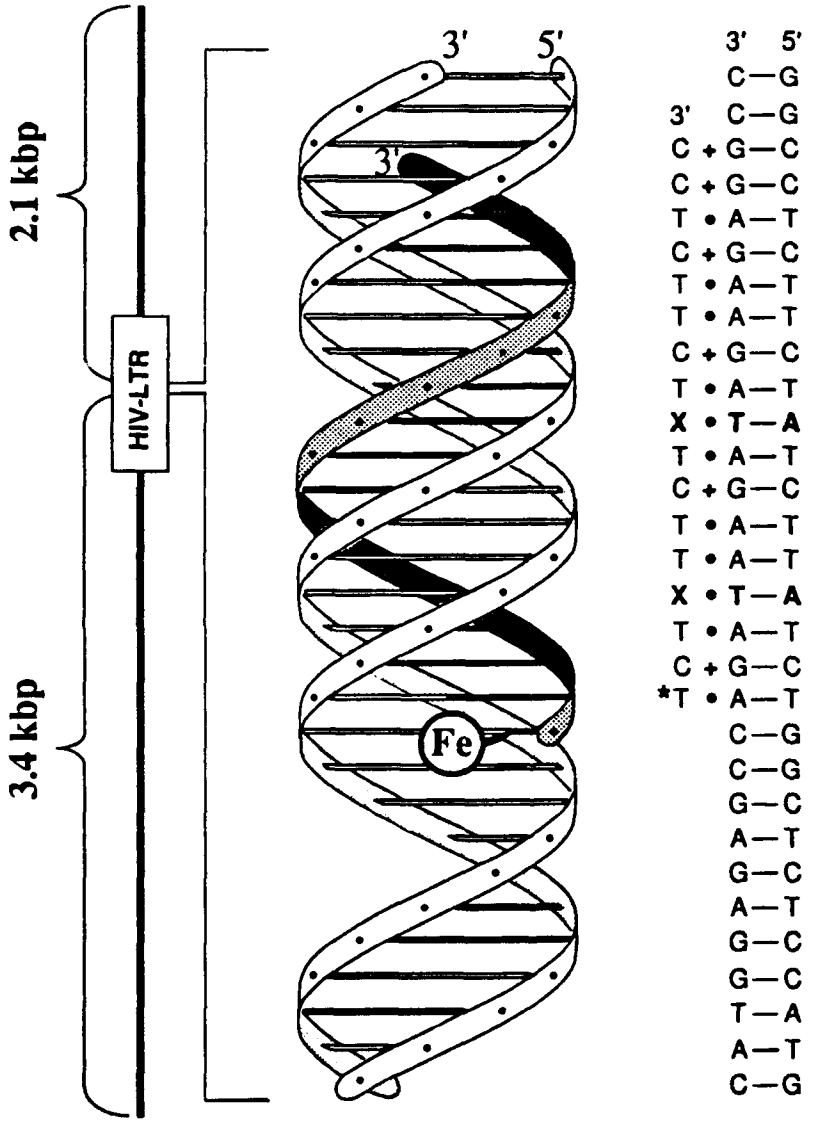

120
100
80
60
40
20
0
Relative % Cleavage
AT
GC
CG
TA
D3
D8
D9
Base

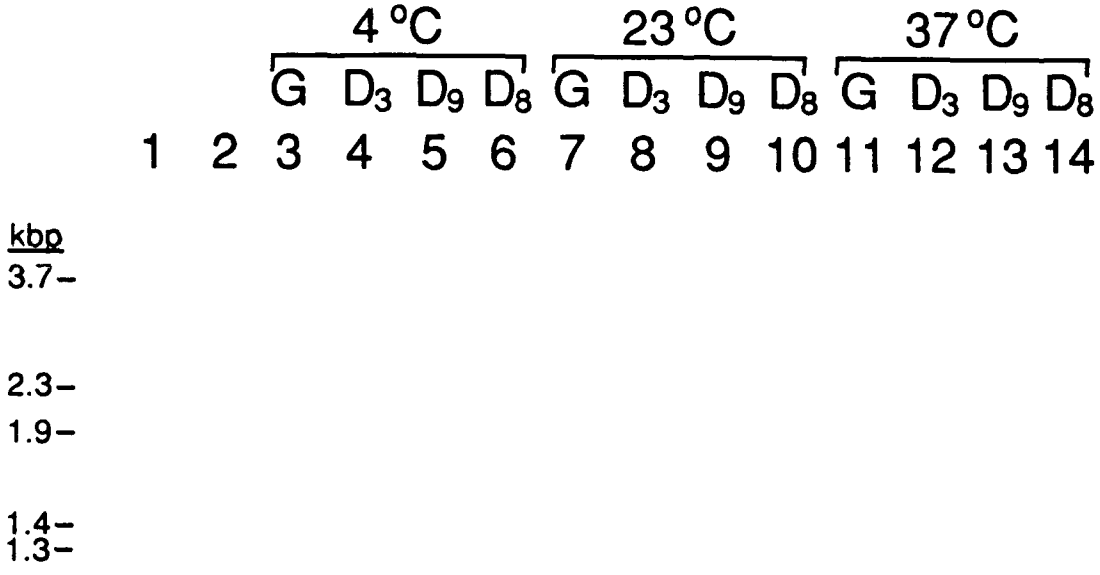
5'- T C T **G** T T C T **G** T C T T C T C C -3' **8**

5'- T C T **D_3** T T C T **D_3** T C T T C T C C -3' **9**

5'- T C T **D_8** T T C T **D_8** T C T T C T C C -3' **10**

5'- T C T **D_9** T T C T **D_9** T C T T C T C C -3' **11**




4 °C
23 °C
37 °C
G D3 D9 D8 G D3 D9 D8 G D3 D9 D8
1 2 3 4 5 6 7 8 9 10 11 12 13 14
kbp
3.7–
–3.4 kbp
2.3–
1.9–
–2.1 kbp
1.4–
1.3–